T4 PDG (T4 Endonuclease V)

2,000 units 10,000 U/ml Lot: 0041305
RECOMBINANT Store at –20°C Exp: 5/15

Description: T4 PDG (pyrimidine dimer glyco-sylase) has both DNA glycosylase and AP lyase activity. The 16 kd protein recognizes cis-syn-cyclobutane pyrimidine dimers caused by UV irradiation. The enzyme cleaves the glycosyl bond of the 5’ end of the pyrimidine dimer and the endonucleolytic activity cleaves the phosphodiester bond at the AP site.

Source: Purified from an E. coli strain carrying a plasmid encoding T4 denV gene

Applications:
- DNA damage studies
- Single cell gel electrophoresis (comet assay)

Reagents Supplied with Enzyme:
10X T4 PDG Reaction Buffer, 100X BSA

Reaction Conditions:
1X T4 PDG Reaction Buffer, supplemented with 100 μg/ml BSA. Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the conversion of 0.5 μg of UV irradiated supercoiled pUC19 DNA to > 95% nicked plasmid in a total reaction volume of 20 μl in 30 minutes at 37°C. Nicking is assessed by agarose gel electrophoresis. Irradiated plasmid contains an average of 3–5 pyrimidine dimers.

Unit Assay Conditions: 1X T4 PDG Reaction Buffer containing 0.5 μg of UV irradiated supercoiled pUC19 DNA, supplemented with 100 μg/ml BSA in a 20 μl reaction.

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

16-Hour Incubation:
- A 50 μl reaction containing 1 μg of λ DNA (Hind III digest) and 100 units of T4 PDG (T4 Endonuclease V) for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 μl reaction containing 100 units of T4 PDG (T4 Endonuclease V) with 1 μg of a mixture of single and double-stranded [3H] E. coli DNA (10^5 cpm/μg) for 4 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

References:

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